

IgG antibodies against microorganisms and atopic disease in Danish adults: The Copenhagen Allergy Study

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Background: Seropositivity to food-borne and orofecal microorganisms (hepatitis A virus, *Helicobacter pylori*, and *Toxoplasma gondii*), which are considered to be markers of poor hygiene, has been reported to be associated with a lower prevalence of atopy. In contrast, colonization of the gut with *Clostridium difficile*, a potential intestinal bacterial pathogen, in early childhood may be associated with a higher prevalence of atopy.

Objective: The objective of this study was to investigate the association between atopy and exposure to 2 groups of food-borne and orofecal microorganisms: (1) markers of a poor hygiene and (2) intestinal bacterial pathogens.

Methods: A cross-sectional population-based study of 15- to 69-year-olds living in Copenhagen, Denmark, was carried out in 1990 to 1991. Atopy was defined as a positive test result for specific IgE to at least 1 of 6 inhalant allergens. Exposure to microorganisms was assessed as IgG seropositivity to microorganisms.

Results: Seropositivity to 2 or 3 markers of poor hygiene (hepatitis A virus, *H pylori*, and *T gondii*) was associated with a lower prevalence of atopy (adjusted odds ratio, 0.5; 95% CI, 0.3 to 0.8). In contrast, seropositivity to 2 or 3 intestinal bacterial pathogens (*C difficile*, *Campylobacter jejuni*, and *Yersinia enterocolitica*) was associated with a higher prevalence of atopy (adjusted odds ratio, 1.7; 95% CI, 1.2 to 2.6).

Conclusion: Exposure to markers of poor hygiene was associated with a lower prevalence of atopy, whereas exposure to intestinal bacterial pathogens was associated with a higher prevalence of atopy. These findings raise the hypothesis that different groups of food-borne and orofecal microorganisms may have different effects on the risk of atopy. (J Allergy Clin Immunol 2003;111:847-53.)

Key words: Allergic rhinitis, atopy, *Campylobacter jejuni*, *Clostridium difficile*, *Helicobacter pylori*, hepatitis A virus, respiratory hypersensitivity, *Toxoplasma gondii*

The prevalence of allergic respiratory disease is increasing in developed countries^{1,2} including Denmark.³ The factors underlying this increase are largely unknown.¹⁻³

Epidemiologic studies have almost consistently shown an inverse relationship between allergic respiratory disease and the number of siblings.⁴ Strachan⁵ hypothesized that the lower prevalence of allergic respiratory disease in large sibships was due to cross-infection between siblings. According to this hygiene hypothesis the increase in the prevalence of atopic disease in developed countries is caused by a decrease in exposure to infections. Most studies on the relationship between infections and allergic respiratory disease have focused on respiratory infections. However, results of studies on the association between respiratory infections and allergic respiratory disease have not been consistent.^{6,7} One study in Italian cadets showed that previous exposure to food-borne and orofecal infections, as reflected by IgG antibodies against hepatitis A virus (HAV), *Helicobacter pylori*, and *Toxoplasma gondii*, was negatively associated with allergic respiratory disease (skin prick test reactivity to inhalant allergens, allergic rhinitis, and asthma).⁸ In contrast, there was no association between IgG antibodies against respiratory viruses and allergic respiratory disease. The authors speculated that seropositivity to food-borne and orofecal infections is the marker of an "unhygienic" lifestyle with a high exposure to pathogens and commensals that stimulate the gut-associated lymphoid tissue and thereby protect against atopy. If these mechanisms actually contribute to the worldwide epidemic of

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Abbreviation used

HAV: Hepatitis A virus

allergic respiratory disease, one would expect the association between allergic respiratory disease and seropositivity to food-borne or orofecal infections observed in Italy to be generalizable to other developed countries. We aimed to investigate the association between these markers of poor hygiene and the prevalence of atopy in a general population in Denmark, a country that differs from Italy with regard to many lifestyle, environmental, and climatic factors.

In contrast to the above microorganisms, exposure to *Clostridium difficile* has been reported to be associated with a higher prevalence of atopic disease in children. Sepp et al⁹ found that fecal samples from 1-year-olds in Sweden, a country with a high prevalence of atopy, were more frequently colonized with *Clostridium* spp, in particular *C difficile*, than those from their peers in Estonia, a country with a low prevalence of atopy. Bottcher et al¹⁰ investigated microflora associated biochemical markers in feces of 13-month-old infants. They found that allergic infants had higher levels of i-caproic acid, which has been associated with the presence of *C difficile*. In a Finnish prospective study, more *Clostridium* spp were detected by using fluorescence in situ hybridization in the feces of 3-week-old infants who developed allergy during their first year of life as compared to those who did not.¹¹ Colonization of the gut with *C difficile* is probably more frequent in persons living in a more "sterile" environment. Accordingly, *C difficile* often colonizes the gut after antibiotic treatment.¹² In this way *C difficile* could be a marker of a certain gut flora that is associated with exposure to a "sterile" environment or diet. Whereas HAV, *H pylori*, and *T gondii* induce chronic infection with little or no alteration of the intestinal flora, *C difficile* may colonize the intestine and have an influence on the intestinal flora. Furthermore, *C difficile* may invade the intestinal mucosa and induce inflammation. The subsequent increased permeability of the mucosal barrier could facilitate the penetration of innocuous antigens. Other intestinal pathogens such as *Campylobacter jejuni* and *Yersinia enterocolitica* may have a similar influence on the intestine.

Thus, the aim of this study was to investigate the association between atopy and 2 groups of food-borne and orofecal microorganisms: (1) a group considered as markers of poor hygiene (HAV, *H pylori*, and *T gondii*) and (2) a group considered as intestinal pathogens (*C difficile*, *C jejuni*, and *Y enterocolitica*).

METHODS**Study population**

The study was conducted according to a 2-stage protocol. In the first stage a screening questionnaire on respiratory symptoms was mailed to a sample of 15- to 69-year-olds (n = 8000) living in the western part of Copenhagen, the capital of Denmark. The sample was drawn randomly from the Civil Registration System. A total of

6998 or 87.5% responded to the screening questionnaire. In the second stage, a random group and a symptom group selected among the respondents were invited to a health examination. The symptom group comprised all respondents (n = 788) who had reported upper ("itchy or stuffy nose or sneezing") or lower ("shortness of breath or trouble breathing") airway symptoms on exposure to either pollen or furry animals in the screening questionnaire.¹³ The random group comprised 647 subjects who were randomly selected from the remaining respondents. Subsequently, 635 (participation rate, 80.6%) and 477 (participation rate, 73.7%) subjects were examined in the symptom and the random groups, respectively. Thus, a total of 1112 subjects (overall participation rate, 77.5%) were examined. The invitational procedure and characteristics of both participants and nonparticipants have previously been described in more detail.¹³ Examinations took place between February 1990 and January 1991. Venous blood was drawn on the day of examination, and the blood samples were left to coagulate for 2 hours. The serum was then separated by centrifugation at 3000 rpm for 10 minutes and frozen at -20°C immediately afterward. The Ethical Committee of Copenhagen County approved the study.

IgE antibodies

Serum samples were analyzed for IgE specific to birch, grass (timothy), mugwort, dog, cat, and mite (*Dermatophagoides pteronyssinus*) in 1999 by using the ADVIA Centaur IgE antibody assay system (Bayer Corporation, New York, NY).³ The analysis for IgE antibodies was judged to be positive if the measurement was in excess of 0.35 kU/L (class I). Furthermore, serum samples were analyzed for total IgE in 1991 by using the Pharmacia CAP System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

IgG antibodies against microorganisms

Commercial assays were used to determine IgG antibody levels in serum to HAV (HAV; Vidas, bioMerieux, Marcy l'Etoile, France), *T gondii* (Vidas, bioMerieux), and *Y enterocolitica* (DAKO, Glostrup, Denmark). Seropositivity was defined according to the manufacturer's instructions. IgG antibody levels in serum to *H pylori*¹⁴ and *C jejuni*¹⁵ were measured by an indirect in-house ELISA previously described in detail. IgG antibody levels in serum to *C difficile* were measured by an indirect ELISA. Microtiter plates (Nunc-Immuno Plate, MaxiSorp; Nunc International, Roskilde, Denmark) were coated overnight at room temperature with a 1:2000 dilution of a *C difficile* antigen solution. The antigen solution was prepared by sonification of a culture of a toxigenic strain of *C difficile* isolated from a patient with pseudomembranous colitis. Protein concentration of the water-soluble antigen was 41.6 g/L. The antigen coating was optimized by checkerboard titration. After washing, serum samples were added in a 1:200 dilution. The assays were done in duplicate with uncoated wells as control for nonspecific binding of Ig or conjugated anti-human Ig rabbit serum to the plastic matrix. After incubation for 1 hour at room temperature the plates were washed, and horseradish peroxidase conjugated rabbit antibodies to human serum IgG (DAKO) diluted 1:2000 were added to each well. The plates were incubated for 1 hour at room temperature and washed, and the enzyme activity was detected by using the orthophenylendiamine dihydrochloride/H₂O₂ system. The chromogenic reaction was stopped with H₂SO₄ after 15 to 30 minutes, and the optical density was read in a photometer at 492 nm. A difference in absorbency of more than 100 between coated and uncoated wells was considered positive. The immunoglobulin content was semiquantified by serial dilution of the samples. The titers were calculated as the lowest dilution with a positive value. All assays were routinely used for clinical samples at the Department of Clinical Microbiology, Rigshospitalet. Cutoff values for seropositivity were

TABLE I. Age-stratified prevalence of IgG seropositivity to microorganisms, atopy, and allergic rhinitis

	15-34 y (total n = 427)	35-49 y (total n = 378)	50-69 y (total n = 296)
	% (n)	% (n)	% (n)
HAV	2.1 (9)	15.9 (60)	51.0 (151)
<i>H pylori</i>	11.9 (51)	27.8 (105)	39.5 (117)
<i>T gondii</i>	22.7 (97)	42.9 (162)	50.7 (150)
<i>C difficile</i>	14.8 (63)	20.9 (79)	23.0 (68)
<i>C jejuni</i>	20.6 (88)	29.4 (111)	32.4 (96)
<i>Y enterocolitica</i>	53.9 (230)	64.3 (243)	70.3 (208)
Index of exposure to markers of poor hygiene*			
0	67.7 (289)	37.8 (143)	20.6 (61)
1	27.9 (119)	41.5 (157)	32.4 (96)
2 or 3	4.4 (19)	20.6 (70)	47.0 (139)
Index of exposure to intestinal bacterial pathogens†			
0	32.3 (138)	20.6 (78)	16.9 (50)
1	48.5 (207)	51.3 (194)	49.7 (147)
2 or 3	19.2 (82)	28.0 (106)	33.4 (99)
Atopy	47.9 (204)	33.2 (125)	23.3 (69)
Allergic rhinitis	36.6 (155)	23.9 (89)	13.5 (40)

*IgG seropositivity to HAV, *H pylori*, and *T gondii* (none, any, and 2 or 3).

†IgG seropositivity to *C difficile*, *C jejuni*, and *Y enterocolitica* (none, any, and 2 or 3).

identical to the values used for clinical samples. IgG measurements were performed in 2001.

Definition of variables

A cumulative index of exposure (IgG seropositivity) to HAV, *H pylori*, and *T gondii* (range: none, any, and 2 or 3) was defined as described by Matricardi et al.⁸ Similarly, a cumulative index of exposure to intestinal bacterial pathogens, *C difficile*, *C jejuni*, and *Y enterocolitica* (range: none, any, and 2 or 3), was defined. Atopy was defined as a positive test result for specific IgE to at least 1 of 6 inhalant allergens. Information on health, lifestyle, and socioeconomic variables was obtained in a questionnaire, which was completed at home.

Allergic rhinitis was defined as at least 1 of the 3 following diagnoses:

1. Allergic rhinitis to pollen: an affirmative answer to the question "Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing when near grass, trees, or flowers?" and a positive test result for IgE antibodies to at least 1 of birch, grass, or mugwort.
2. Allergic rhinitis to animals: an affirmative answer to the question "Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing following exposure to furry animals, eg, horse, dog, cat, rabbit, guinea pig, or hamster?" and a positive test result for IgE antibodies to at least 1 of dog or cat.
3. Allergic rhinitis to mite: an affirmative answer to the question "Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing following exposure to dust when cleaning rooms or making beds, or when in bed?" and a positive test result for IgE antibodies to mite.

The following potential confounders were considered: sex, age (15 to 34, 35 to 49, 50 to 69 years), educational level (9 or less, 10 to 11, 12 to 13, 14 or more years), smoking status (never, previously, currently), family history of hay fever, dog in household (never, previously, currently), cat in household (never, previously, currently), number of siblings (0, 1, 2, 3, 4 or more siblings), daycare attendance (never, 1 to 2 years before age 5, 3 to 5 years before age 5), and increased total IgE level (100 kU or greater).

Statistical analysis

The primary and secondary outcomes (dependent variable) were atopy and allergic rhinitis, respectively. Associations between seropositivity to microorganisms and the dependent variable were expressed as odds ratios with 95% confidence intervals. The confounding effect of other covariates was evaluated in multivariate logistic regression analysis. Both indexes of exposure and all covariates were included as categorical variables, and dummy variables were created for comparing each level to a reference level. A test for trend¹⁶ was performed to evaluate a possible dose-response relationship between each index of exposure and atopy (or allergic rhinitis). A difference in the mean number of siblings between the categories of each index of exposure was tested by Kruskal-Wallis test. The independent associations between the indexes of exposure and total IgE were evaluated by linear regression with log total IgE as the dependent variable and the indexes of exposure, atopy, age, sex, and smoking as the independent variables. All data were analyzed with SPSS for Windows (Release 10.0; SPSS, Inc, Chicago, Ill).

RESULTS

The prevalence of IgG seropositivity against each of the microorganisms increased with increasing age (chi-square test, $P < .05$) (Table I). The prevalence of atopy was negatively associated with seropositivity to HAV and positively associated with seropositivity to *C difficile* (Table II). Furthermore, the prevalence of atopy decreased with an increasing index of exposure to markers of poor hygiene (HAV, *H pylori*, and *T gondii*). In contrast, the prevalence of atopy increased with an increasing index of exposure to intestinal bacterial pathogens. The effect of these indexes of exposure remained significant when they were both included in a multivariate logistic regression model including potential confounders (Table III and Fig 1). In this model there was no statistically significant interaction between the 2

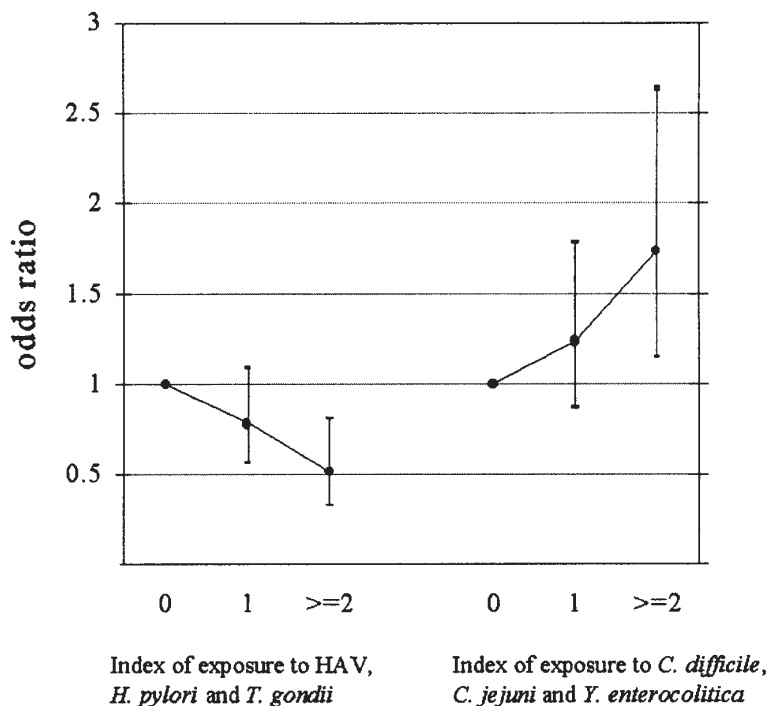


FIG 1. Adjusted odds ratios and 95% CIs for the association between the prevalence of atopy and cumulative indexes of exposure to markers of poor hygiene (HAV, *H pylori* and *T gondii*) and intestinal bacterial pathogens (*C difficile*, *C jejuni*, and *Y enterocolitica*). Risk estimates are obtained in a logistic regression model with adjustment for age, sex, smoking, increased total IgE, number of siblings, and family history of hay fever.

indexes of exposure or between age and each of the 2 indexes of exposure. However, the association between atopy and the index of exposure to intestinal bacterial pathogens tended to be stronger in the youngest part of the population. Thus, the adjusted odds ratio for the association between seropositivity to 2 or 3 intestinal bacterial pathogens was 3.63 (95% CI, 1.84 to 7.19), 1.28 (95% CI, 0.61 to 2.67), and 1.39 (95% CI, 0.53 to 3.64) among 15- to 34-year-olds, 35- to 49-year-olds, and 50- to 69-year-olds, respectively. This difference between age groups was not statistically significant (P value for test for interaction between the index of exposure to intestinal bacterial pathogens and age was 0.177). The adjusted odds ratio for the association between seropositivity to 2 or 3 markers of poor hygiene was 0.34 (95% CI, 0.09 to 1.27), 0.53 (95% CI, 0.25 to 1.08), and 0.58 (95% CI, 0.25 to 1.32) among 15- to 34-year-olds, 35- to 49-year-olds, and 50- to 69-year-olds, respectively. Essentially similar results were seen with allergic rhinitis as the dependent variable.

The risk estimates for the indexes of exposure changed little when age was included as a continuous variable in the above analyses.

Of note is that the sibling effect was not explained by exposure to markers of poor hygiene or intestinal bacterial pathogens (Table III). There was not any significant interaction between the number of siblings and each index of exposure. The mean number of siblings did not

differ significantly ($P = .469$) between the categories of the index of exposure to intestinal bacterial pathogens. In contrast, the mean number of siblings differed significantly ($P = .001$) between the categories of the index of exposure to markers of poor hygiene. Thus, the mean number of siblings increased with increasing exposure to markers of poor hygiene.

The associations between the prevalence of allergic rhinitis and IgG seropositivity to microorganisms were essentially similar (Tables II and III).

Increased total IgE could be speculated to be an intermediate variable in the relationship between exposure to microorganisms and the development of atopy. However, excluding total IgE from the regression models did not change the estimates substantially. In linear regression analysis, the indexes of exposure were not significantly associated with total IgE.

DISCUSSION

We found a negative association between a cumulative index of exposure to HAV, *H pylori*, and *T gondii* and the prevalence of atopy and allergic rhinitis. This is consistent with findings of a nested case-control study in Italian military cadets, aged 17 to 24 years.⁸ Thus, this association is not due to factors specific to Italian lifestyle, environment, or climate. In contrast, a nested case-control study (85 cases with adult onset wheeze and 190 con-

TABLE II. The association between IgG seropositivity to microorganisms and the prevalence of atopy (specific IgE positivity) and allergic rhinitis

		Atopy		Allergic rhinitis	
		Prevalence % (n)	Odds ratio*	Prevalence % (n)	Odds ratio*
Markers of poor hygiene					
HAV	No	40.2 (353)	1	29.4 (257)	1
	Yes	20.5 (45)	0.57 (0.38-0.86)	12.3 (27)	0.58 (0.36-0.95)
<i>H pylori</i>	No	39.2 (323)	1	28.7 (236)	1
	Yes	27.5 (75)	0.78 (0.57-1.08)	17.7 (48)	0.74 (0.51-1.07)
<i>T gondii</i>	No	40.6 (280)	1	29.8 (205)	1
	Yes	28.9 (118)	0.76 (0.57-1.00)	19.5 (79)	0.75 (0.55-1.04)
Index of exposure†	0	44.8 (220)	1 <i>P</i> = .002‡	33.3 (164)	1 <i>P</i> = .007‡
	1	34.5 (128)	0.80 (0.60-1.08)	24.3 (90)	0.80 (0.58-1.11)
	2 or 3	21.2 (50)	0.52 (0.35-0.79)	12.8 (30)	0.51 (0.32-0.83)
Intestinal bacterial pathogens					
<i>C difficile</i>	No	35.5 (315)	1	25.2 (223)	1
	Yes	39.5 (83)	1.42 (1.03-1.98)	29.3 (61)	1.51 (1.06-2.16)
<i>C jejuni</i>	No	36.4 (293)	1	26.1 (209)	1
	Yes	35.7 (105)	1.15 (0.86-1.55)	25.7 (75)	1.18 (0.85-1.64)
<i>Y enterocolitica</i>	No	36.5 (153)	1	27.5 (114)	1
	Yes	36.1 (245)	1.09 (0.84-1.44)	25.1 (170)	1.00 (0.74-1.34)
Index of exposure§	0	35.3 (94)	1 <i>P</i> = .033‡	28.1 (74)	1 <i>P</i> = .087‡
	1	36.1 (197)	1.24 (0.89-1.71)	23.9 (130)	0.95 (0.67-1.36)
	2 or 3	37.3 (107)	1.50 (1.03-2.18)	28.1 (80)	1.42 (0.95-2.12)

*Odds ratio (95% CI) for the association between atopy (or allergic rhinitis) and seropositivity to different microorganisms adjusted for sex, age, and family history of hay fever.

†IgG seropositivity to HAV, *H pylori*, and *T gondii* (none, any, and 2 or 3).

‡*P* value for test for trend.

§IgG seropositivity to *C difficile*, *C jejuni*, and *Y enterocolitica* (none, any, and 2 or 3).

trol subjects) in a general population aged 34 to 40 years in Scotland did not find an association between atopy (skin prick test reactivity to inhalant allergens) and seropositivity to HAV, *H pylori*, and *T gondii*.¹⁷ Also this study did not find an association between atopy and number of siblings.¹⁷ One recently published study in a large population-based sample in the United States could confirm the negative association between seropositivity to HAV and *T gondii* and atopy (skin prick test reactivity to inhalant allergens and peanut), self-reported hay fever, and asthma.¹⁸ Thus, the negative association between these microorganisms and allergic respiratory disease seems to exist in regions as diverse as North America,¹⁸ southern Europe (Italy),^{8,19} and northern Europe (Denmark). Seropositivity to HAV, *H pylori*, and *T gondii* is considered to be a marker of an “unhygienic” (or original) lifestyle with a high exposure to pathogens and commensals that stimulate the gut-associated lymphoid tissue, thereby protecting against atopy. This lifestyle includes naturally prepared foods (without the use of preservatives and pasteurization). It should be noted that a mechanism for a direct protective effect of HAV against the development of atopy has been suggested.²⁰

There is evidence of a marked decrease in seroprevalence of HAV,²¹⁻²⁵ *H pylori*,²⁶ and *T gondii*^{27,28} during recent decades. The marked decrease in seroprevalence of these microorganisms with decreasing age (Table I) observed in the present study supports that a decrease in exposure to these microorganisms has also occurred in

Denmark. The decrease in seroprevalence of these microorganisms appears to have coincided with the increase in atopic disease, lending support to the hypothesis that improved hygiene has contributed to the epidemic of atopic disease.

We found a positive association between atopy and seropositivity to intestinal bacterial pathogens, which is in contrast to the negative association between atopy and seropositivity to markers of poor hygiene. The reasons for this discrepancy are not clear. Whereas HAV, *H pylori*, and *T gondii* induce chronic infection with little or no alteration of the intestinal flora, intestinal bacterial pathogens may colonize the intestine and induce changes in the composition of the intestinal flora. Moreover, the ability of intestinal pathogens to invade the intestinal mucosa with subsequent inflammation may increase the permeability of the mucosal barrier, facilitating the penetration of innocuous allergens, which could hamper the maintenance of oral tolerance. Several studies have suggested that the composition of the intestinal flora plays a role in the induction and maintenance of oral tolerance to innocuous antigens.^{7,29} Bjorksten et al³⁰ found that 2-year-old children with allergy were less often colonized with *Lactobacillus* spp and *Bifidobacterium* spp and harbored higher counts of aerobic bacteria (coliforms, *Staphylococcus aureus*) than children without allergy. In a further prospective study they observed that infants who had developed allergy at 2 years were less often colonized with enterococci during the first month of life and

TABLE III. Results of multivariate analysis of the relationship of markers of poor hygiene and intestinal bacterial pathogens to the prevalence of atopy and allergic rhinitis

Covariate	Dependent variable	
	Atopy Odds ratio*	Allergic rhinitis Odds ratio*
Index of exposure to markers of poor hygiene†		
0	1 <i>P</i> = .005‡	1 <i>P</i> = .006‡
1	0.78 (0.56-1.09)	0.77 (0.55-1.10)
2 or 3	0.51 (0.32-0.81)	0.48 (0.28-0.81)
Index of exposure to intestinal bacterial pathogens§		
0	1 <i>P</i> = .009‡	1 <i>P</i> = .052‡
1	1.24 (0.87-1.78)	0.86 (0.59-1.26)
2 or 3	1.74 (1.15-2.64)	1.54 (1.00-2.39)
No. of siblings		
0	1 <i>P</i> = .006‡	1 <i>P</i> = .010‡
1	0.99 (0.64-1.54)	0.95 (0.60-1.51)
2	0.72 (0.45-1.14)	0.80 (0.50-1.31)
3	0.71 (0.40-1.26)	0.48 (0.25-0.92)
≥4	0.52 (0.29-0.95)	0.57 (0.29-1.12)
Family history of hay fever		
No	1	1
Yes	1.67 (1.21-2.29)	2.15 (1.55-2.98)

*Odds ratios (95% CI) adjusted for variables shown in the table including sex, age, smoking status, and increased total IgE (≥ 100 kU).

†IgG seropositivity to HAV, *H pylori*, and *T gondii* (none, any, and 2 or 3).

‡*P* value for test for trend.

§IgG seropositivity to *C difficile*, *C jejuni*, and *Y enterocolitica* (none, any, and 2 or 3).

with *Bifidobacterium* spp during the first year of life.³¹ The administration of *Lactobacillus* prenatally and postnatally to high risk infants may prevent the development of atopic eczema.³² Furthermore, intestinal colonization with enterobacteria such as *Escherichia coli* of newborns in Sweden occurs later than in their peers in developing countries.²⁹ The delayed colonization of the intestine of Swedish children with Enterobacteriaceae spp may facilitate colonization with other bacteria such as *Clostridium* spp.²⁹ Thus, *C difficile* could be a marker of a “Westernized” or “un-natural” gut flora. Wold²⁹ hypothesized that the overly hygienic lifestyle in modern Western societies has altered the normal intestinal colonization pattern in infancy, and that this has led to a failure to induce and maintain oral tolerance of innocuous antigens. Moreover, a high turnover of different bacteria genera, species, and strains may be important for the induction and maintaining of tolerance.⁷

Assuming that early childhood or adolescence is the period at which susceptible persons are at the highest risk of developing atopy, exposure to intestinal bacterial pathogens at older ages may not be associated to the same extent with the development of atopy. This may be a possible explanation for the finding that seropositivity to intestinal bacterial pathogens was mainly associated with atopy in the youngest part of the population. Detectable serum IgG against chronic infections (HAV, *H pylori*, and *T gondii*) probably persists lifelong. As for acute infections, Strid et al³³ found that the population mean serum IgG after verified *Campylobacter* enteritis decreased during the first 4 to 5 months and thereafter was reasonably stable during a 2-year follow-up period.

Loss of detectable IgG against intestinal pathogens could possibly account for the finding that the association between atopy and seropositivity to intestinal bacterial pathogens appeared to be stronger in the youngest part of the population.

IgE and IgG antibodies were measured in serum samples that had been stored for 8 to 10 years. We have previously confirmed the stability of IgE antibodies in serum samples that had been stored at –20°C for 8 years.³

As described above, participants were selected in 2 groups, a random group and a symptom group. This could potentially induce bias. Including a group variable (symptom versus random group) in the regression models presented in Table III did not change the estimates of the other variables substantially. Also there was not any significant interaction between the group variable and the indexes of exposure to microorganisms included in the models.

To our knowledge this is the first study to report an association between seropositivity to intestinal bacterial pathogens and atopy; therefore further studies are warranted. Furthermore, the interpretation of cross-sectional data may be difficult. However, the present study has raised the hypothesis that intestinal bacterial pathogens may have an influence on the development of atopy. Thus, different groups of food-borne and orofecal infections may have different effects on the development of atopy.

Our data are consistent with previous reports that have linked *C difficile* to atopic disease.⁹⁻¹¹

The interpretation of the well-established epidemiologic association between atopy and the number of siblings is still controversial.^{4,6,34} It is of note that in the present population the sibling effect was independent of

markers of exposure to food-borne or orofecal infections (Table III). This may suggest that the sibling effect is not mediated by cross-infection by these microorganisms. However, microorganisms other than those included in the present analyses may mediate the sibling effect. Alternatively, the protective effect of infections explaining the sibling effect could take place in utero and therefore might not be followed by increased IgG antibody levels against microorganisms in adulthood.

In conclusion, we found that exposure to a group of microorganisms that are considered to be markers of poor hygiene was associated with a lower prevalence of atopy. In contrast, exposure to a group of microorganisms that are considered to be intestinal pathogens was associated with a higher prevalence of atopy. These findings suggest that different groups of food-borne and orofecal infections may have different effects on the risk of developing atopy.

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